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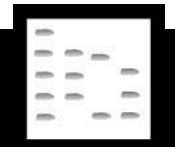
CLINICAL CHEMISTRY: THIN-LAYER (PLANAR) CHROMATOGRAPHY



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Introduction

Clinical medicine is mainly based on the results of chemical analysis and these results are very important. It is estimated that more than 50% of diagnoses are based on laboratory data. The analyses are usually done using immunoassays, chromatographic and spectroscopic methods. The combined techniques, e.g. liquid chromatography–mass spectrometry (LC/MS) gas chromatography–tandem mass spectrometry (GC-MS-MS) are also applied. The development of sophisticated methods of analyses reduced interest in simple and rapid methods such as thin-layer chromatography (TLC). Starting from paper chromatography and improved over the years, TLC has become very useful also for clinical medicine. TLC has been used to solve analytical, and sometimes preparative, problems.

A large number of publications on the use of TLC for clinical medicine analysis can be found in the literature. An excellent compilation concerning different applications of analytical methods (including TLC) for isolation and identification of drugs in pharmaceutical, body fluids and post-mortem materials is the book edited by Moffat. This work, prepared in the Department of Pharmaceutical Sciences of the Pharmaceutical Society of Great Britain, is a comprehensive and clear account of clinical analytical chemistry up to 1986. An excellent supplement, covering the early 1990s, is the monograph of Jain. More recent works more commonly refer to applications of instrumental TLC.

General Principles of Clinical Medicine Analyses by TLC

TLC is a most economical and cost-effective technique and ideally suited for performing clinical analyses of biological samples, where the sample load is high. The method is characterized by high selectivity, enabling separation of the analyte from interfering substances. At least two basic groups of applications of TLC in clinical medicine analysis can be distinguished. The most important of them are where TLC has been applied for analytical and screening purposes where qualitative or semi-quantitative results are required. The second group concerns work, where TLC performs the function of a clean-up technique for initial sample preparation.

Clinical analysis presents a difficult analytical problem. The proteins, lipids, or carbohydrates, usually present in biological samples, may interfere with analytes. Analyses are also made difficult by the fact that many substances of interest are only slightly soluble in organic solvents.

Sample Preparation

There is a generally accepted view in laboratory practice, that the TLC stage of sample preparation is of little importance because it is merely a clean-up technique. This is incorrect, especially in the case of clinical medicine analysis where analytes are to be found in complex matrices such as plasma, serum or urine, whole blood, faeces, saliva, cerebrospinal fluid, gastric fluid or body tissues. These matrices are very complex multicomponent mixtures and therefore sample preparation prior to analysis cannot be omitted.

The process of separation from biological samples and purification of analytes is usually realized by protein precipitation, dialysis, hydrolysis, ultrafiltration, dilution, liquid–liquid or solid-phase extraction. Originally, the most common extraction method was

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liquid–liquid extraction but in recent years solid-phase extraction has become an increasingly popular method for isolation of compounds from biological matrices. Lyophilization, saponification, microwave processing and supercritical fluid extraction are used less commonly.

Chromatogram Development Techniques

Chromatographic separation of clinical samples is carried out mainly in normal phase systems. High performance silica gel, aluminium oxide, polyamide and amino-bonded silica gel are commonly used as stationary phases for the separation of basic and acidic drugs and other polar substances such as amino acids. It is better to separate neutral drugs and other nonpolar compounds in reversed-phase (RP) systems. The commonest stationary phases for RP systems are silica gel with chemically bonded octyl and octadecyl groups. Numerous solvent systems consisting of mixtures of organic solvents such as hexane, methanol, chloroform, ethyl acetate or mixtures of polar solvents with water, organic acids or ammonia have been used. It is sometimes advantageous to introduce modifying agents to the mobile phase to improve selectivity.

One-dimensional ascending or horizontal techniques have usually been applied for the development of chromatograms in a closed chamber. For clinical medicine needs, there are standard TLC kits (Toxi-Prep type and Toxi-Lab). These are commercially available and have been used above all for extraction, concentration and analysis of the acidic, basic, and neutral drugs. Recently, multiple gradient development (AMD), two-dimensional techniques and overpressure layer chromatography have been more often applied to the analyses listed in **Table 1**.

Visualization and Quantification

Identification of analytes is achieved by co-chromatography of reference standards with the unknown and comparison of R_F values. TLC retention indices are less reliable than for other chromatographic methods so that additional information from individual spots is needed. Post-chromatographic derivatization is the basic method of visualization especially when there exists a specific reaction that can confirm the presence of particular analytes in the spot or band. Physical methods such as fluorescence under ultraviolet light are also often applied. The possibility of confirmation of identity based on at least two criteria is the essential advantage of TLC. The colorimetric reactions of drugs, narcotics, and other substances of clinical interest with different visualization reagents are listed in **Table 2**. Identification can also be made using spectroscopic techniques such as TLC/FABMS or FABMS-MS (fast atom bombardment mass spectrometry or tandem mass spectrometry).

Densitometric measurement of the *in situ* fluorescence or absorbance provides information about the quantity of an analyte in a chromatographic band. Such measurements are suitable for the quantification of drugs such as chlorpromazine, tricyclic antidepressant and anticonvulsant in blood serum and plasma. Quantitative analyses are also performed by combination of TLC with other analytical techniques, e.g. UV-densitometry.

Applications

In a view of the complexity of clinical analysis, it is not possible to cover all applications extensively. Therefore, only the most important applications of TLC in clinical medicine analyses are presented. The criteria of aims of analysis are: (1) the diagnosis and treatment of hard intoxication, (2) screening research, (3) monitoring of therapeutic drugs concentration, and (4) evaluation of the efficiency of new methods of therapy. In such applications, the drugs and their metabolites, carbohydrates, amino acids, bile acids, lipids, porphyrins and other compounds of clinical interest are analysed. The results of such work are collected in libraries (databases) that significantly facilitate diagnosis and therapy.

The Diagnosis and Hard Intoxication Treatment

The term hard intoxication is applied to the determination of harmful effects, arising in a short time after introducing a large dose of poison to the body. These effects are mainly observed after drugs or alcohol overdose or the introduction of organic solvents, pesticides, etc., into body. The symptoms of intoxication can be similar to those caused by disease so that diagnosis based only on clinic symptoms in insufficient; in such situations analytical data are very important. Only laboratory results, can determine the type and concentration of poison in a biological material or a functional disorder caused by overdose intoxication or poisoning. The biological samples, where poisons and/or their metabolites are found are mainly blood or urine. The most important features of such analyses are the necessity to determine the type of poison as quickly as possible. The basic requirement (especially in analysis followed by diagnosis) is for information about the poison, from simple, rapid and reliable qualitative or semiquantitative methods. The common use of TLC in clinical medicine analyses of hard intoxication is due to its ability to provide such data.

Table 1 The applications of thin-layer chromatography (TLC) in clinical medicine research. The representative examples of separations

No.	*Matrix and analyte*		*Chromatographic system*		*Type of development*
	Analyte	*Body fluid*	*Stationary phase*	*Mobile phase*	
1	Theophyline	Serum	Aminopropylsiloxane modified silica	Ethanol–5% aqueous diethylamine solution (95 : 5)	One-dimensional, isocratic
2	Tetrahydro-cannabinol (THC) metabolites	Urine	HPTLC silica gel	SPE elution solution: ethyl acetate–concentrated ammonium hydroxide (98 : 2) Developing solution: heptane–ethyl acetate–glacial acetic acid (7 : 3 : 10)	Toxi-Prep system: incorporate SPE with direct spotting onto TLC plate, one-dimentional, isocratic elution
3	Bile acid	Bile	Silica gel	Chloroform–methanol–acetic acid (85 : 20 : 9)	One-dimensional, isocratic
4	Porphyrins	Urine, faeces	RP-HPTLC (C-18 bonded wettable phase)	Acetonitrile–ion-pair reagent (IPR)–acetate buffer pH4,1 (9 : 5 : 5) IPR = *N*-cetyl-*N*,*N*,*N*-trimethylammonium bromide	One-dimensional, isocratic
5	Amino acids	Urine	Cellulose	(I) Pyridine–acetone–aqueous ammonium hydroxide–water (13 : 8.5 : 2.5 : 6) (II) Isopropanol–formic acid–water (25 : 3 : 2)	Two-dimensional, isocratic
6	Amphetamine derivatives	Urine	Aminopropylsiloxane modified silica	(I) Ethanol–triethylamine–hexane (15 : 9 : 76) (II) Acetone–triethylamine–hexane (23 : 9 : 68)	Two-dimensional, isocratic
7	Lipids	Sebaceous gland	Silica gel with concentrating zone	Hexane–diethyl ether–acetic acid (80 : 20 : 1)	One-dimensional, isocratic
8	Prostaglandins	Serum	HPTLC silica gel	Ethyl acetate–diethyl ether–benzene–dioxane–hexane (45 : 12 : 5 : 8 : 30)	OPLC one-dimensional
9	Amino acid derivatives	Urine	Silica gel	Chloroform–methanol–ethyl acetate–acetic acid	AMD gradient elution
10	Barbiturate derivatives	Serum, urine	HPTLC silica gel	(I) Ethyl acetate–ethanol–hexane (2.5 : 2.5 : 95) (II) Pyridine–hexane (2 : 8)	OPLC two-dimensional
11	Tetrahydro-cannabinol (THC)	Urine	HPTLC I-silica gel WRFs II-silica gel F	(I) Dichloromethane–hexane–methanol (7 : 2 : 1) (II) Dichloromethane–hexane–methanol (7 : 1 : 2)	One-dimensional, isocratic
12	Ecdysteroids	Plasma	RP-TLC (C-2,C-8, C-12, C-18, aminopropyl, cyanopropyl bonded phases)	Methanol–water (9 : 1)	One-dimensional, isocratic

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The majority of this work is aimed at the determination of analytical parameters of xenobiotics and/or their metabolites. In diagnostic tests using TLC, identification is based mainly on comparison of R_F values and on visualization with specific colour reactions. Tests for the identification of alkaloids,

Table 2 Examples of visualization and quantification

Analyte	*Visualization method*	*Quantification*	
		Yes	*No*
Amino acids			
Common protein amino acids	3-Phenyl-2-thiohydantoin (PTH) derivative, 270 nm absorption		X
Urinary amino acids (incl. proline and hydroxyproline)	Ninhydrin, isatin, isatin-*p*-dimethylaminobenzaldehyde		X
Bile acids	Manganese dichloride in a mixture of water, methanol and sulfuric acid and fluorescent measurement		X
Cholesterol and its esters	Aniline blue, bromophenol blue, helasol green and alkaline blue	X	
Drugs			
Antibiotics			
Doxycycline, oxytetracycline and tetracycline	Fluorescent measurement, 365 nm /> 440 nm	X	
Aminoglycoside streptomycin and neomycin	Dansylation and fluorescent measurement	X	
Baclofen	Dansylation and fluorescent measurement,	X	
Barbiturates	Mercuric chloride-diphenylcarbazone reagent; fluorescein solution; mercurous nitrate spray		X
Benzodiazepines	UV radiation 254/366 nm, dipped in concentrated sulfuric acid and observed under UV (366 nm); diazotization and coupling with 1-naphthol or Bratton–Marshall reagent and next fluorescent measurement	X X	
β-Blocking drugs			
Atenolol, celiprolol, metoprolol, propafenone, propranolol and talinolol	Brillant Blue B, UV light absorption (254 nm)	X	
Halofantrine	UV light absorption (256 nm)	X	
Nonsteroidal anti-inflammatory drugs (e.g. ketoprofen, piroxicam, diclofenac, ibuprofen, paracetamol)	UV light absorption (254 nm)		X
Narcotics			
Amphetamine metabolites	UV light absorption (278, 283 nm) and by fluorescence after derivatization with fluorescamine 365 nm/ > 440 nm	X	
Heroin (diacetylmorphine)	$HgCl_2$–$K_3Fe(CN)_6$ and light absorption (580 nm)		X
Δ^9-Tetrahydrocannabinol (THC)	UV light absorption (210 nm)	X	
Tricyclic antidepressive drugs Fluoxetine, imipramine, doxepine, opipramol	Amelinka's reagent or tested by UV irradiation	X	
Phospholipids	Iodine vapour		X
Free porphyrins	Fluorescent measurement	X	
Prostaglandins	α-Bromo-2′-acetonaphthone (BAN) and fluorescent measurement	X	

barbituric acid derivatives, benzodiazepine derivatives and other drugs from other pharmacological groups have been known for many years.

Mathematical methods have recently been applied in the diagnosis of hard intoxication. The first step of these methods is to evaluate the R_F values in two, three and sometimes four chromatographic systems. These systems must be carefully selected to ensure an appropriate distribution of R_F values across the plates, the reproducibility of the measurement of these parameters and the correlation of chromatographic properties between systems. Next, the sets of correlated R_F values are determined (experimentally determined R_F values are converted into correlated R_F values by a graphical standardization procedure) in a possibly large group of substances. In case of clinical analyses there are usually hundreds of drugs and their metabolites. A mathematical function (e.g. 'discriminating power' or 'principal component analysis') for calculation of analytical data is then chosen. The data set obtained allows for relatively rapid identification of investigated (unknown) xenobiotics. A full description of the application of such methods can be found in the work by de Zeeuw *et al*.

TLC as a Screening Method

Screening methods reduce the cost and time of analysis of numerous groups of samples. In clinical

medicine analysis, this term is referred to a set of biological samples (laboratory screening methods) or to a set of individuals endangered by contact with harmful substances (metabolic screening). In both cases, the aim of analysis is the exclusion of a particular sample (for an individual) from the set of samples (individuals), which should be examined with great care. Application of TLC to such aims is attractive as this technique enables simultaneous analysis of several samples.

Laboratory screening methods are similar to diagnostic analyses, but the object of investigation is usually known. During the screening other properties such as changes in the body under xenobiotics influence, e.g. changes in enzyme activity, creation of methemoglobine, increased porphyrin expulsion, etc. are measured. Because drugs or their metabolites are excreted and concentrated in urine, this is the matrix most commonly used for such examination. Especially useful for screening investigations are kits such as the Toxi-Prep (TP) kit, proposed by Steinberg and coworkers which can simultaneously extract up to seven specimens. The method is based on TLC and involves five major steps: solid phase extraction, concentration, spotting, development of chromatograms and detection.

The kits have been found to be particularly useful for analysis of basic and neutral drugs. The comparison of the usability of three kits (TL-A, TL and TP) in the monitoring of basic drugs is presented in **Table 3**.

Another example of screening methods is the detection of an unknown, potentially toxic xenobiotic in the presence of a number of endogenous substances. Such defined screening methods are focused on limiting the number of expensive methods of analyses.

Metabolic screening is based on observation of changes in biochemical profile of patients within a relatively short time. The commonest analyses are for cholesterol and lipids or amino acids and porphyrins. An example of such an application of TLC is the work of Lai *et al*. They proposed a simple test for the determination of porphyrins (porphyrins play a crucial role in the biological processes of haem synthesis; in the case of defects in the biosynthetic

Table 3 Overall urine basic drug analysis[a]

Drug	*Occurrences on*		
	Both TP and TL	*TP only*	*TL-A only*
Amitryptyline and metabolites	5	4	2
Caffeine		0(1)	
Cimetidine		1(1)	
Clindamycin and metabolites	2		4
Codeine		1	
Cyclobenzaprine		1	
Desipramine and metabolites	1	1	
Diphenhydramine	5(3)	1(7)	
Doxepin		1	
High migrator	13	3	5(3 = cocaine; 2 = lidocaine)
Imipramine	1		
Low migrator		0(1)	
Meperidine and metabolites		1(1)	1
Methadone and metabolites	16(1)		5
Metoprolol			1
Morphine	0(1)	0(2)	6
Nicotine and metabolites	17	2	34
Nortriptyline and metabolites	16	10(2)	2
Oxycodone		1	
Phenotiazine	3		1
Quinidine/quinine	46	8(5)	
Ranitidine	2		
Sympathomimetic amines	2(2)	0(1)	
Trazodone metabolites	2(1)		
Verapamil	2	2	
Unidentified substance		1	3
Totals	141	59	64
(33 distinct drugs)	[133(8)][b]	[38(21)][b]	[64][b]

[a]Reprinted from Steinberg DM *et al.* (1997) *Clinical Chemistry* 43(11): 2099–2105, with permission from the American Association of Clinical Chemistry.

[b]Number of times definitively identified (questionable identifications).

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pathway of haem an increase of porphyrin excreted is observed). Numerous groups of people are occupationally exposed to the substances causing porphyries, and these have to be periodically screened. The urinary porphyrin-free acids are separated on a RP-HPTLC plate coated with C_{18} bonded silica gel. The mobile phase is buffered (pH 4.1) with a ternary mixture (acetonitrile, ammonium acetate buffer, ion-pairing reagent). Porphyrins, separated by TLC, create spots with a characteristic profile (fingerprints), and these profiles (differing in the quantity of excreted porphyrins) are observed in porphyria (**Figure 1**). By observing chromatograms in UV light, the basic criteria of metabolic screening can be fulfilled.

TLC has the advantage over other methods in that it can screen for many drugs simultaneously, with a relatively high sensitivity, and can handle several samples per plate.

Drug Concentration Monitoring

The pharmacological action of many drugs depends not on the amount taken but on the concentration in the blood. Relations between these two values (dose and therapeutic concentration) depend on the drug and often have individual characteristics. It was found that after taking the same dose of phenytoin (drug in anticonvulsants) its concentration in patients blood differed more than twentyfold. Therefore, one of the tasks of clinical medicine analyses is to establish relationships between the concentration of the drug in blood and its dosage. Such analyses are called drug concentration monitoring. They are performed mainly on the dosage of drugs of little therapeutic value, combined therapy, etc. Drug concentration monitoring requires use of relatively simple, cheap and rapid methods, which indicates the special suitability of TLC for such analysis. Drug concentration monitoring requires (in contrast to diagnostic and screening methods) quantitative analysis.

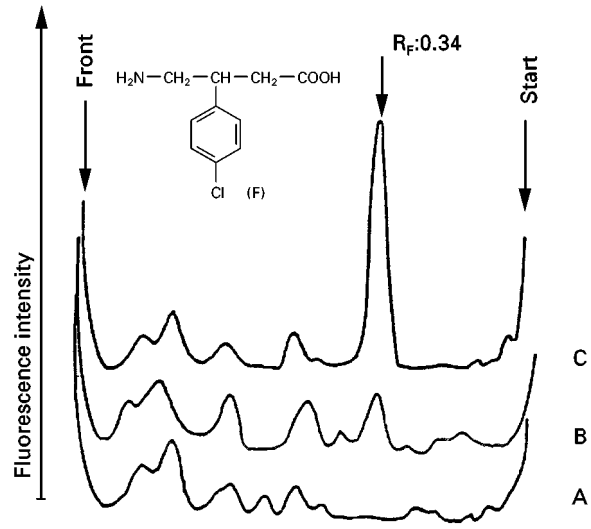

Figure 2 Representative TLC chromatograms of (A) a blank plasma sample, (B) derivatization product of baclofen after extraction from volunteer plasma (70 ng baclofen mL^{-1}), (C) derivatization product of the fluoro analogue after extraction from volunteer plasma (320 ng of the fluoro analogue mL^{-1}). Reprinted from Krauss D, Spahn H and Mutschler E (1988) *Arzneimittel Forschung Research* 38(II)(10): 1533–1536, with permission.

A good example of the application of TLC for drug concentration monitoring is the work by Krauss *et al.* on baclofen and its fluoro analogue (these are centrally acting muscle relaxants, which are used for the treatment of spastic disorders). Gas chromatography with electron-capture detection requires a time-

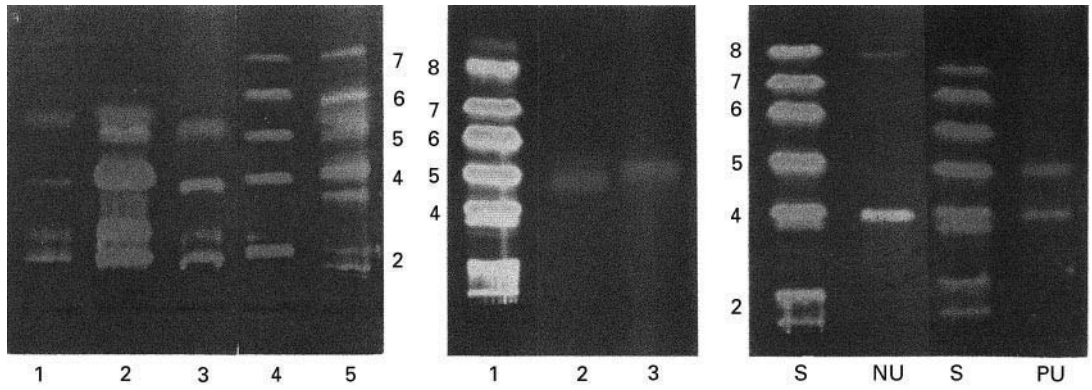

Figure 1 (See Colour Plate 69). RP-HPTLC chromatograms of fecal porphyrins of porphyria cutanea tarda (PCT) and variegate porphyria (VP) patients. Left panel: lane 1, VP feces of patient 1; lane 2, VP feces of patient 2; lane 3, external quality control sample of VP; lane 4, mixed calibrators of free porphyrins; lane 5, PCT feces; d, deuteroporphyrin. Middle panel: lane 1, mixed calibrators of free porphyrins; lane 2, i-urobilin standard; lane 3, stercobilin standard. Right panel: S, mixed calibrators of free porphyrins; NU, commercial urine control with a negative Urobilistix result; PU, urine obtained from a patient with hepatitis and a positive Urobilistix result; 8, uroporphyrin; 7, heptacarboxylic porphyrin; 6, hexacarboxylic porphyrin; 5, pentacarboxylic porphyrin; 4, coproporphyrin, 3, tricarboxylic porphyrin; 2, protoporphyrin. Reprinted from Lam C-W, Lai C-K and Chan Y-W (1998) *Clinical Chemistry* 44 (2): 345–346, with permission from the American Association of Clinical Chemistry.

consuming separation and derivatization step; HPLC does not require derivatization but the detection limit is insufficient for such studies. Several enantiospecific methods have also been developed but their applicability to biological material has not been shown. The method proposed by Krauss is based on fluorescent derivatization of the drug with benoxaprofen chloride, TLC separation of the resulting amides and their quantification by direct measurement of the fluorescence. This method requires extensive purification of the samples (e.g. solid-phase extraction) in order to remove interfering endogenous amino acids. Chromatograms are obtained with a stationary phase of silica gel 60 without a fluorescence indicator (plates with concentrating zones) and a mobile phase of diisopropyl ether–2-propanol–tetrahydrofuran, 90 : 6 : 5, v/v. After development, both compounds were separated from constituents of the biological material (**Figure 2**). The fluorescence intensity of the derivatization products is sufficient to determine plasma concentrations over a suitable period of time after single dose administration of both drugs. The detection limit (10 ng mL^{-1}), linearity (60 ng $spot^{-1}$) and method deviation have been estimated. The applicability of the method has been proved by investigating plasma (and urine) samples of two volunteers after oral administration of 20 mg baclofen as a single dose. Full suitability of the method for pharmacokinetic routine analyses was demonstrated.

Evaluation of the Efficiency of New Methods of Therapy

Evaluation of the efficiency of new methods of therapy mainly concerns drugs newly introduced into

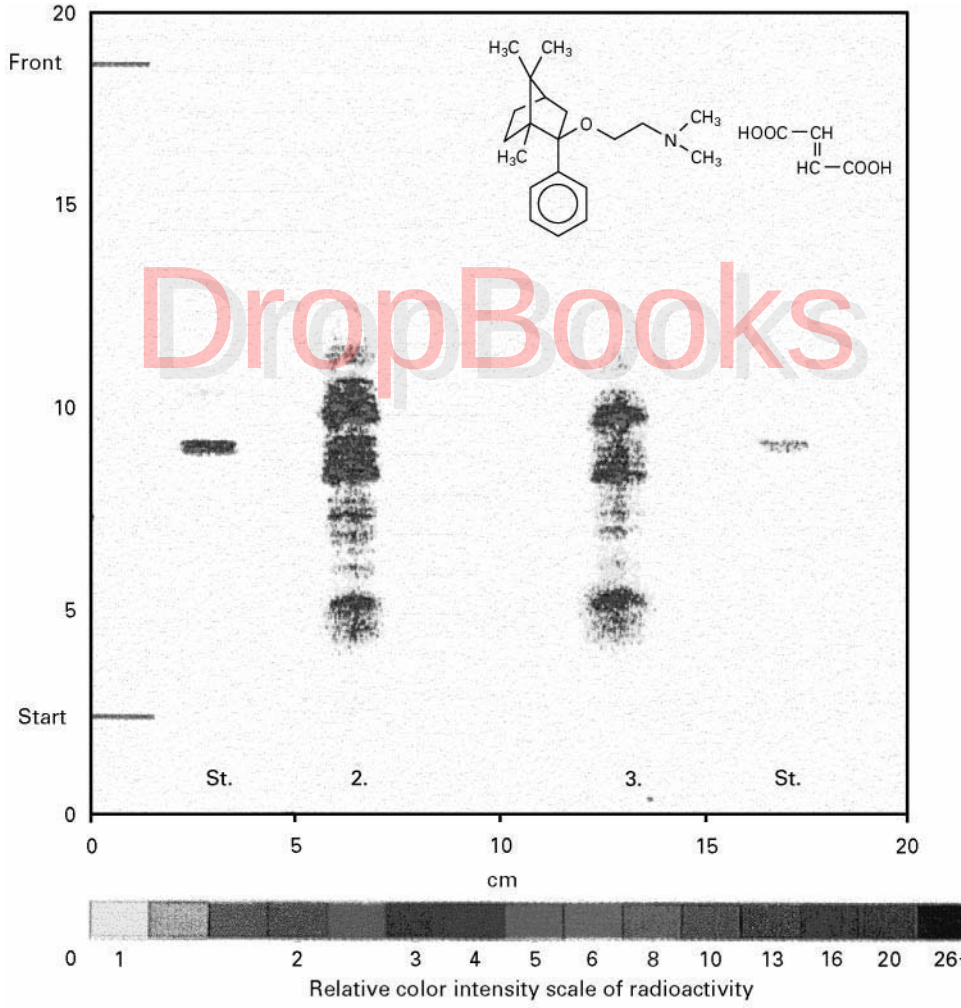

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Figure 3 (See Colour Plate 70). OPLC–DAR profile of dog urine extracts. Sample, dog urine samples after 10 mg kg^{-1} oral dosing of ^{14}C-daramciclane. The first and fourth tracks are standards (St); tracks 2 and 3, 0–24-h and 24–48-h urine fractions, respectively. Single OPLC development eluent A; external pressure, 5.0 Mpa; flow rate, 250 μL min^{-1}, rapid volume, 250 μL; eluent volume, 4100 μL; time, 994 s; DAR run time, 60 min. Reprinted from Szúnyog J, Mincsovics E, Hazai I and Klebovich I (1998) *Journal of Planar Chromatography–Modern TLC* 11 (1): 25–29, with permission.

clinical medicine. From an analytical point of view it is a more complicated process than drug concentration monitoring since the object of investigation is not only concentration of the drug in blood but also its assimilation and excretion, harmfulness, metabolism, etc. Such investigations are performed mainly on blood and urine samples.

An interesting example of abilities of TLC in the above applications is the work of Szúnyog *et al*. They applied a combination of overpressure-layer chromatography (OPLC) with the relatively high sensitive and rapidity of digital autoradiography (DAR). Biological samples were analysed after one- or two-dimensional separation. Using the example of deramciclane (a new anxiolytic compound) they demonstrated, that the proposed method could be successfully used for study of *in vivo* metabolism in different phases of studies. The main advantages of the method were the extremely rapid separation and purification by OPLC, the possibility of the quantitative evaluation of metabolites over a wide linear range, visual and numeric comparison of metabolite profiles in various biological matrices and cost-effective metabolism studies (**Figure 3**).

TLC as a Clean-up Technique

The basic method of applications of TLC as a clean-up technique consists of the preparative separation of analytes and removal from the TLC plate together with adsorbent and elution with an appropriate solvent. This technique is used mainly in pharmacological work. In clinical medical analyses the method is used to separate mixtures and then introduce the fractions obtained to other analytical instruments. From the analytical point of view, in these methods TLC has the role of a clean-up technique.

In analytical practice two basic methods of TLC combined with other analytical methods are applied. The older method of off-line coupling is similar to preparative cleaning of the sample. Eluate obtained after chromatographic separation and sorbent separation is introduced into the analytical instrument. The technique should be used when the recovery of an analyte is of secondary importance. An advantage of off-line methods is the possibility of performing analysis with optional detectors. In on-line methods the TLC plate is introduced to the detector step-by-step. Analytes are removed from the adsorbent by cathode sputtering or laser desorption. An important advantage of such methods is the full quantitative analysis of practically all substances of clinical interest. Unfortunately, these techniques are expensive and require very specific injectors that are not usually commercially available.

Martin *et al*. applied TLC coupled with MS–MS to the analysis of antipyrine and its metabolites in extracts of human urine. The urine samples were obtained from a volunteer who had received a single oral dose of antipyrine. After 12 h, an aliquot was enzymatic hydrolysed to liberate the analytes from glucuronide conjugates. Next, analytes were extracted (LLE), concentrated and separated by TLC. A good separation (**Figure 4**) was obtained on silica gel HPTLC plates with a mobile phase of chloroform–methanol–trifluoro-acetic acid, 95 : 5 : 1, v/v.

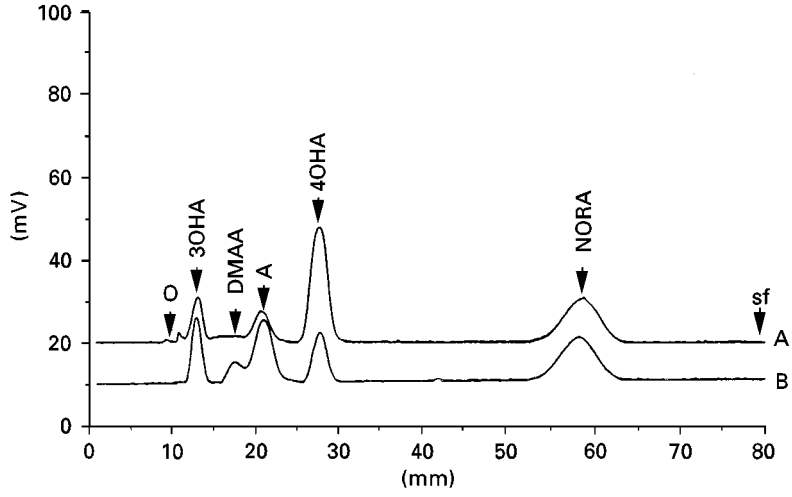

Figure 4 Chromatograms obtained from (A) a urine sample following enzymic hydrolysis and solvent extraction and (B) a standard mixture of the analytes and the internal standard: o, origin; sf, solvent front; 3OHA, 3-hydroxyantipyrine; DMAA, dimethylaminoantipyrine; A, antipyrine; 4OHA, 4-hydroxyantipyrine; NORA, norantipyrine. Reprinted from Martin P, Morden W, Wall P and Wilson I (1992) *Journal of Planar Chromatography–Modern TLC* 5(4): 255–258, with permission.

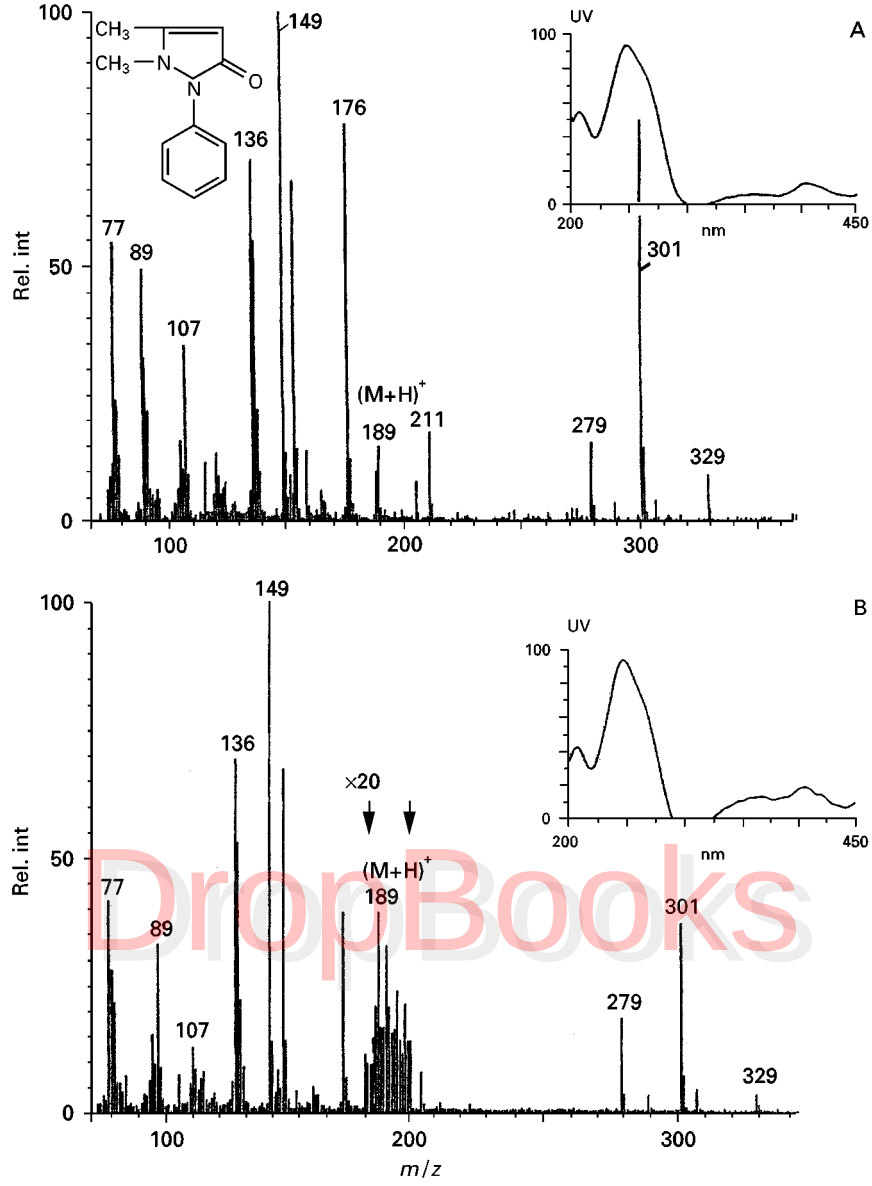

Figure 5 TLC–FABMS spectra obtained directly from the silica gel for (A) antipyrine and (B) material co-chromatographing with antipyrine from the urine extract (insets: structure and UV spectra). Reprinted from Martin P, Morden W, Wall P and Wilson I (1992) *Journal of Planar Chromatography–Modern TLC* 5(4): 255–258, with permission.

The appropriate areas of the plate were then removed for further analysis by FAB MS and FAB MS–MS. The authors demonstrated (**Figure 5**) that this relatively simple technique would appear to make TLC–FAB MS–MS eminently suitable for confirming the identity of drug metabolites in body fluids for the study of drug metabolism or the detection of drugs abuse, etc.

Conclusions

The analytical tasks connected with clinical needs are very large. It is estimated that the annual increase in the number of such analyses is about 15–20% with systematic increases in the number of different clinical chemical parameters. However, not all clinical analyses can be performed using TLC. TLC is relatively simple, comes with low equipment cost, and has both a high efficiency and sensitivity. Some disadvantages of TLC include only fair specificity and the requirement of skill in accurately recognizing drug patterns by interpreting the coloured spots visualized with selective reagents, etc. It should also be emphasized that TLC is a comparative method; more complicated tasks in clinical medicine (investigation of metabolism and metabolite

structure) can be performed by this method but only in combination with other, mainly spectroscopic, analytical techniques.

See Colour Plates 69, 70.

See also: **II/Chromatography:** Paper Chromatography. **Chromatography: Gas:** Detectors: Selective; Gas Chromatography–Mass Spectrometry. **Chromatography: Liquid:** Detectors: Mass Spectrometry. **Chromatography: Thin-Layer (Planar):** Densitometry and Image Analysis; Layers; Mass Spectrometry; Modes of Development: Conventional; Modes of Developement: Forced Flow, Over Pressured Layer Chromatography and Centrifugal; Spray Reagents. **Extraction:** Analytical Extractions; Solid-Phase Extraction; Solvent Based Separation; Supercritical Fluid Extraction. **III/Amino Acids:** Thin-Layer (Planar) Chromatography. **Bases: Thin-Layer (Planar) Chromatography. Bile Compounds: Thin Layer (Planar) Chromatography. Biomedical Applications:** Gas Chromatography-Mass Spectrometry; Thin-Layer (Planar) Chromatography. **Carbohydrates:** Thin-Layer (Planar) Chromatography. **In-Born Metabolic Disorders: Thin-layer (Planar) Chromatography. Lipids:** Thin-Layer (Planar) Chromatography. **Proteins:** Thin-Layer (Planar) Chromatography.

Further Reading

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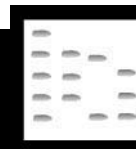
CLINICAL DIAGNOSIS: CHROMATOGRAPHY



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Clinical laboratories within the UK are responsible for providing services for the diagnosis and monitoring of disease. There are several specialities within clinical laboratories or pathology: haematology – examining blood cells and factors relating to blood cell production; biochemistry – measurement of metabolites, hormones, drugs, proteins; histopathology — examination of tissues and cells, usually microscopically; immunology – assessment of antibody status in disease; molecular biology and cytogenetics – specialist services looking at genetic disease. Of these departments, the clinical biochemistry repertoire is the most amenable to chromatography.

Clinical biochemistry laboratories in an average district general hospital perform over a million analyses per annum. The large volume of samples (average request/analysis ratio $\sim 1:4$) received each day, the clinical demand for rapid turnaround plus the need for analytical imprecision of less than 5% with acceptable relative accuracy means that high levels